

# Metal Speciation in Environmental and Biological Systems

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Metal ions form complexes with naturally occurring complexing agents or ligands released from industrial activity. The metal complexes are thereby mobilized and transported in environmental and biological systems. The impact of such metal complexes depends on the metal complex species that are kinetically and thermodynamically stable in these homogeneous and heterogeneous systems. The distribution of metal complex species in these complex systems can be calculated from available formation constant data. This article describes the importance of verifying the validity of these calculations by employing experimental techniques that can speciate metal complexes. — *Environ Health Perspect* 103(Suppl 1):13–16 (1995)

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## Introduction

Many thousands of organic compounds are produced every year by industrialized countries. These compounds as well as their byproducts eventually find their way into the environment where they can undergo biologic, oxidation–reduction, or photochemical reactions. It is expected, therefore, that some fraction of these organic compounds will be converted into metal-binding ligands. It is estimated that the number of these potential ligands will increase 1 to 2% each year as a consequence of the development of new products. Roughly one-third of the elements in the periodic table can react with organic ligands. The elements of most concern are those that are abundant and are extracted and used in various chemical industries. It is expected that these elements will perturb biogeochemical cycles and that humans will eventually be exposed to these elements or their compounds.

It is not surprising that organic ligands introduced into the environment that have appropriate functional groups can mobilize and transport certain elements by complex formation. The term “complex formation” is used here in its broadest sense, i.e., the successive formation of metal–ligand complexes such as  $\text{ML}$ ,  $\text{ML}_2$ ,  $\text{ML}_3$ , etc. (where M and L with charges omitted, represent

the metal ion and ligand, respectively), the formation of mixed ligand complexes (e.g.,  $\text{MLX}$ ,  $\text{MLY}$ , etc. where X and Y represent auxiliary ligands), in homogeneous systems and, in heterogeneous systems, the formation of adsorption complexes with particulate matter or with cell surfaces (Figure 1). The presence of these complexes in the environment is governed by their thermodynamic stability, i.e., their formation constants and by their rates of formation and dissociation at concentrations that are found in the environment, and not under experimental conditions that are convenient for laboratory studies.

Of urgent concern are the complexes formed by some of the heavier elements in the periodic table: arsenic, selenium, cadmium, indium, antimony, tellurium, mercury, thallium, lead, and bismuth. Evidence indicates that these heavy elements and their compounds or complexes have been mobilized and transported in air, surface waters, in sediments and in the soil,

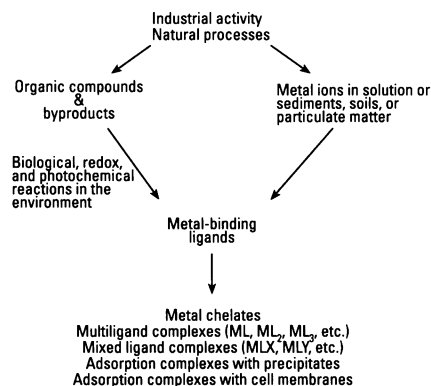
and have, therefore, significantly perturbed the environment. Moreover, their intake by humans via food, water, and particulates in the air have caused health concerns (1). It is important to determine experimentally or to deduce from the body of published data, the nature of these heavy element complexes. The bioavailability and toxicity of these heavy element complexes are critically dependent on their thermodynamic and kinetic stability. Identifying these species (i.e., speciation) and obtaining reasonable estimates of their thermodynamic and kinetic stabilities are the problems that continue to plague environmentalists and toxicologists.

## Speciation in Homogeneous and Heterogeneous Systems in the Environment

### Homogeneous Systems

Among the group of heavy elements that can have potential health effects, the more commonly encountered heavy elements, mercury, lead, and cadmium, have been selected to illustrate the general approaches that have proved useful in their speciation. The conditions under which these speciations are performed are highly simplified and do not reflect the complex conditions that are usually found in environmental systems. The emphasis in the examples that will be described is on environmental systems rather than biological systems.

In the majority of instances, the nature of the heavy element complexes that may be present in the environment has to be inferred and their concentrations have to be calculated; there are very few direct experimental methods that are available for speciation. A large database of formation



**Figure 1.** Mobilization and transport of metal complex species.

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constants and rate constants is essential, therefore, for making meaningful deductions about the relative concentrations of heavy element species that may be present in the environment. Such comprehensive databases do not exist; currently, a database of several thousand formation constants of metal complexes that may be found in the environment is available. Many rate constants for the formation of metal complexes have been experimentally determined, but kinetic data for the formation and dissociation of adsorption complexes are almost nonexistent.

In the absence of experimental values of formation constants, it is useful, at least as first approximations, to estimate the formation constants of complexes formed with metals and ligands that have been introduced into the environment as a result of industrial activities or natural processes. Many empirical and theoretical approaches have been used to estimate formation constants. In groups of closely related ligands, unknown formation constants can be calculated from linear free energy relationships, for example, between  $\log K_{ML}$  and  $\log K_{HL}$  where  $K_{ML}$  is the formation constant of the metal complex  $ML$  and  $K_{HL}$  is the protonation constant of the ligand  $L$ . Other linear relationships have also proved to be useful, e.g., between  $\log K_{ML}$  and  $e^2/r$  (where  $e$  and  $r$  are the charge and radius of the metal ion), and between  $\log K_{ML}$  and the electronegativities of the metal ions (2,3). More recently, empirical four-parameter and six-parameter equations (4,5) based on thermodynamic cycles have been employed to calculate the free energy, enthalpy, and entropy of complex formation in solution. These calculations depend on the availability of the thermodynamic quantities for the corresponding gas-phase reaction and for the desolvation of the reactants and

solvation of the products. In the absence of such thermodynamic quantities, these empirical approaches are of limited use. Obviously, the ideal method for the calculation of unknown metal complex formation constants involves a detailed understanding at the atomic and molecular levels of the factors that control metal complex formation. It will not be possible, however, to perform such calculations in the foreseeable future. In the absence of reliable theoretical methods for the calculation of unknown values of metal complex formation constants, the estimation of these values by comparison with analogous complex formation reactions has proven to be sufficiently accurate for most environmental systems. The method of using estimated values of metal complex formation constants is illustrated in the following example in which the distribution of mercury species in seawater is determined by the concentrations of monodentate anions, polydentate complexing ligands, the pH, and the oxidation potential of seawater. Estimated values of the complex formation constants of mercury (II) have been corrected for the high ionic strength of seawater (Table 1). The calculations have been simplified by ignoring the effects of temperature and pressure on the equilibria. It has been assumed that the ionic strength of seawater is approximately 0.7 and is controlled by  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  ions; the pH is 8.1, the  $pK_w$  is 13.8, and hence the pOH is 5.7 (all concentrations are expressed in moles/liter). Seawater may also contain low concentrations of certain organic complexing agents such as humic and fulvic acids that have been carried into the sea by rivers and man-made pollutants such as nitrilotriacetic acid (NTA). These complexing agents have large metal complex formation constants, and it is likely that most trace metals in

seawater are strongly bound to these organic complexing agents. Again, to simplify the calculation of species distribution, it will be assumed that the seawater does not contain any organic complexing agents.

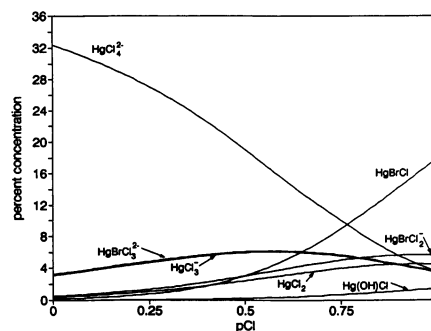
In the oxidizing conditions that prevail in seawater, the stable oxidation state of mercury is +II. It has been well established that mercury (II) is methylated in aquatic systems to the methylmercury cation,  $\text{CH}_3\text{Hg}^+$ , and the volatile dimethylmercury,  $(\text{CH}_3)_2\text{Hg}$ , which is very slightly soluble in water (6,7). The methylmercury cation forms a very stable ion association complex,  $\text{CH}_3\text{HgCl}$ , with chloride ion and it has been shown experimentally that approximately 60% of the total mercury in seawater exists as this stable methylated species (8), which does not dissociate to any appreciable extent, and which does not form complexes with additional halide ions; the concentration of the dimethyl species in aqueous systems is negligible.

Mercury (II) ion and the complexing anions that are present in seawater interact to form labile complexes and equilibrium is established rapidly. The distribution of the mercury (II) species can be calculated from the corresponding formation constants. The values of the constants, however, are not known in seawater, but they can be estimated from values that have been measured in similar media (9). Table 1 shows the formation constants of the mercury complexes that are applicable in a solution of ionic strength 0.7. If the free ligand concentrations in seawater are:  $[\text{OH}^-] = 2.0 \times 10^{-6} \text{ M}$ ;  $[\text{CO}_3^{2-}] = 6.3 \times 10^{-5} \text{ M}$ ;  $[\text{Br}^-] = 7.9 \times 10^{-4} \text{ M}$ ;  $[\text{SO}_4^{2-}] = 1.3 \times 10^{-2} \text{ M}$ ; and  $[\text{Cl}^-] = 0.56 \text{ M}$ ; the calculated fractional concentrations (expressed as percentages) of the labile mercury (II) complexes are shown in Figure 2 as a function of chloride ion concentration. It is not

**Table 1.** Estimated complex formation constants of selected mercury(II), lead(II), and cadmium(II) species in seawater (10).

$\text{Hg}^{2+}$ species	$\log \beta_n$	$\text{Pb}^{2+}$ species	$\log \beta_n$	$\text{Cd}^{2+}$ species	$\log \beta_n$
$\text{Hg}(\text{OH})^+$	10	$\text{Pb}(\text{OH})^+$	7	$\text{Cd}(\text{OH})^+$	3.6
$\text{HgCl}^+$	6.7	$\text{PbCl}^+$	17	$\text{CdCl}^+$	1.4
$\text{HgCl}_2$	13	$\text{PbCl}_2$	17	$\text{CdCl}^+$	1.8
$\text{HgCl}_3^-$	14	$\text{PbCl}_3^-$	27	$\text{CdCl}_3^-$	1.4
$\text{HgCl}_4^{2-}$	15	$\text{PbCO}_3$	24	$\text{CdBr}^+$	1.6
$\text{HgBr}^+$	9			$\text{CdBrCl}$	2.3
$\text{HgBrCl}$	15.9				
$\text{HgBrCl}_2^-$	16.4				
$\text{HgBrCl}_3^{2-}$	17.2				
$\text{HgBr}(\text{OH})$	17.4				

$\beta_n$  (where  $n=1-4$ ) represent cumulative formation constants.



**Figure 2.** Distribution of mercury(II) species in seawater calculated on the basis that approximately 60% of the mercury(II) is present as  $\text{CH}_3\text{HgCl}$ .

surprising that of the labile mercury (II) complexes in seawater, the major component is the  $\text{HgCl}_4^{2-}$  species that predominates at the high chloride ion concentration that is found in seawater (0.56 M). The estimated formation constants for the ion association complexes  $\text{Hg}(\text{OH})^+$ ,  $\text{HgSO}_4$ , and  $\text{HgCO}_3$  indicate that these species are not formed in seawater (10).

It must be emphasized that the results shown in Figure 2 for the distribution of mercury (II) species in seawater are highly oversimplified, and unless all complexing ligands present in the medium are identified, erroneous conclusions could be drawn. For example, the presence of certain organisms in seawater may result in the formation of very stable mercury (II) complexes with ligands containing sulfur donor atoms, possibly from mercapto groups. In the oxidizing environment of the seawater, the oxidation of the mercapto compounds to the disulfides will release the mercury (II) ions that will eventually form the halide complexes shown in Figure 2. Therefore, the species distributions calculated from the metal complex formation constants are the final states toward which the kinetically controlled systems will proceed.

Similar calculations have been used to plot the distribution of lead and cadmium species in seawater as a function of chloride ion concentration (Figures 3,4). The estimated complex formation constants employed in the calculations are shown in Table 1. The principal lead species present at a chloride concentration of 0.56 M is  $\text{PbCl}_3^-$ , followed by the ion association complex  $\text{PbCO}_3$  and the rest of the chloride complexes  $\text{PbCl}_2$  and  $\text{PbCl}^+$ . Interestingly, even at this high chloride ion concentration, there is a significant concentration of  $\text{Pb}(\text{OH})^+$  and uncomplexed  $\text{Pb}^{2+}$ . It may be inferred from Figure 3 that the  $\text{PbCO}_3$  species will predominate in freshwater. Of course, this is on the assumption that strong organic complexing agents are not present either in seawater or in freshwater.

In Figure 4, the principal cadmium species present in seawater at a chloride ion concentration of 0.56 M are the chloro-complexes  $\text{CdCl}_2$ ,  $\text{CdCl}^+$ , and  $\text{CdCl}_3^-$ . The concentration of uncomplexed cadmium increases as expected with a decrease in the concentration of chloride ion.

### Heterogeneous Systems

The environment is a heterogeneous system on a macroscopic as well as a microscopic scale, and it follows that environmental chemistry is mostly a study of heteroge-

neous systems. It has been estimated that the major fraction of metals in "solution" in aquatic systems are in reality adsorbed on solid surfaces of colloids or in particulate matter (11). There is general agreement that in the presence of realistic concentrations of anionic complexing agents and in the absence of strong complexing agents such as nitrilotriacetic acid (NTA) and fulvic acid, the major species of lead found in freshwater is the ion-association complex  $\text{PbCO}_3$  (12).

The calculations of species distributions in these heterogeneous systems present formidable problems. The equilibria that are established between dissolved metal complexes and metal complexes that are adsorbed on colloidal materials or particulate matter are governed in most instances by unknown equilibrium constants. Unless a thorough understanding of the nature of these colloidal and particulate surfaces at the atomic and molecular levels is achieved, the manner in which these surfaces interact with dissolved species cannot be predicted. Information on species distribution in heterogeneous systems must await the development of new and innovative experimental methodology.

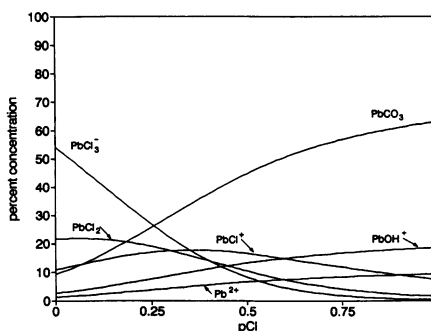


Figure 3. Distribution of lead(II) species in seawater.

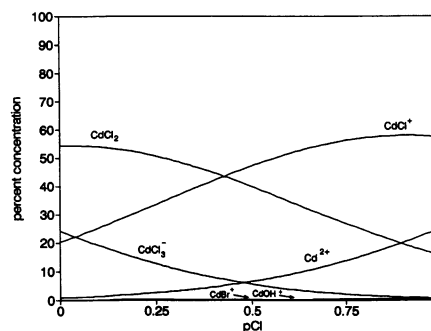


Figure 4. Distribution of cadmium(II) species in seawater.

### Experimental Problems in Speciation

There are many multielement techniques for the determination of total trace element concentrations (at parts per million and parts per billion levels) in environmental and biological samples. The choice of method depends on many considerations. In the analysis of biologic samples, for instance, important factors are that the amount of sample is usually very limited and stringent precautions must be taken to prevent contamination of such samples in the course of sample collection and preparation for analysis. If there are no problems associated with sample storage, it is obviously advantageous to employ a nondestructive analytical method. In biological samples, total element profiles are more meaningful than a single-element analysis because the interpretation of the analytical results will be influenced by changes in the concentrations of all the elements in the sample. Additional considerations are sensitivity and the effects of the presence of interfering elements in the sample.

Some of the common methods that have been employed for the determination of parts per million to parts per billion levels of metals in environmental and biologic samples are atomic absorption methods with a flame or graphite furnace, atomic emission with an inductively coupled plasma, electrochemical methods such as anodic or cathodic stripping voltammetry, high performance liquid chromatography, gas liquid partition chromatography, and X-ray fluorescence. In the last 10 to 15 years there has been a renewed interest in the X-ray fluorescence method as a result of the availability of Van de Graaff accelerators that have been replaced in physics laboratories by higher energy accelerators. High energy proton beams (~2 Mev) from a Van de Graaff accelerator, when used as the incident radiation on environmental or biologic sample targets, yield X-ray spectra with greatly reduced background radiation. This results in a significantly enhanced sensitivity over X-ray fluorescence methods in which other types of incident radiation such as X-rays or electrons, are employed. The most important advantage of this proton-induced X-ray fluorescence technique (PIXE) is that it can be used to determine multielement concentrations in a solid sample as small as 0.1 mg or a single drop of a liquid sample that is evaporated on an inert backing to form a thin uniform film. The analysis time for determining approximately 20 to 30 elements is about 5 min; the method is essentially nondestructive and the spectrum acquisition and data

processing can be completely automated (11,12). Additionally, the proton beam can be focused and areas from  $10\text{ }\mu\text{m}^2$  to  $100\text{ }\mu\text{m}^2$  can be analyzed for heavy elements (micro-PIXE) present at parts per million concentration levels.

The methods described above are capable of determining total trace element concentrations, which is only the first step in the complete characterization of an environmental or biologic sample. The next, more difficult, step is the speciation of the elements that are present. The speciation of compounds stable in the gas phase can be accomplished by a chromatographic separation and the use of a mass spectrometer as a compound-specific detector (i.e., GC-MS). Speciation of soluble compounds can also be performed by HPLC-MS. The success of these speciation methods depends heavily on the availability of standards of the compounds being speciated.

The speciation of labile metal complexes by a direct experimental technique is a difficult problem because the experimental method must not disturb the equilibria that have been established in solution. Indirect

methods such as potentiometry with ion-selective electrodes may be used to deduce the nature of the metal complex species that are present in solution. The few direct methods that may prove to be useful such as multinuclear nuclear magnetic resonance and Raman spectroscopy lack sufficient sensitivity to probe solutions containing trace levels of metal complex species.

Speciation in heterogeneous systems presents serious difficulties. There is a dearth of kinetic data and equilibrium constant data on reactions that occur at interfaces, especially solid-solution interfaces. Moreover, the experimental techniques, such as electron spectroscopy, for the investigation of solid surfaces must be employed under vacuum and, therefore, are of little value in providing information on environmental problems. A better theoretical understanding of the complex chemical reactions occurring at solid-solution interfaces of systems found in the environment must await the development of experimental methods that can probe these interfaces.

In addition to the problems inherent in the speciation of metal complexes found in

environmental and biological samples, there are severe problems that must be overcome in the collection and preservation of these samples prior to analysis. The following example emphasizes the difficulties encountered in sample handling in the course of the determination of arsenic species at parts per billion levels in aqueous solutions. Determining concentrations of arsenite and arsenate in natural waters is of special interest. There is no reliable method, however, that can be used to preserve the initial integrity of the sample and to prevent the interconversion of these two arsenic species. The arsenite concentration was found to first increase with time and then decrease; the rate of decrease was found to depend on the presence of trace components in the sample. It is of vital importance, therefore, to determine trace (parts per million-parts per billion) concentrations of species in a complex environmental or biologic sample as a function of time and to take appropriate steps whenever possible to preserve the integrity of the sample.

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